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Fatty acid composition of wild mushroom species of order Agaricales—Examination by gas chromatography–mass spectrometry and chemometrics☆

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ABSTRACT

Applying gas chromatography–mass spectrometry of 4,4-dimethyloxazoline fatty acid derivatives, the fatty acid composition of 15 mushroom species belonging to 9 genera and 5 families of order Agaricales growing in Bulgaria is determined. The structure of 31 fatty acids (not all present in each species) is unambiguously elucidated, with linoleic, oleic and palmitic acids being the main components (ranging between 70.9% (*Marasmius oreades*) and 91.2% (*Endoptychum agaricoides*)). A group of three hexadecenoic positionally isomeric fatty acids, 6-, 9- and 11–16:1, appeared to be characteristic components of the examined species. By applying chemometrics it was possible to show that the fatty acid composition closely reflects the classification of the species.

1. Introduction

The kingdom of Fungi is assumed to comprise about one million species. Of these, "the true mushrooms", the Basidiomycetes, are estimated to excess 100,000. About 25,000 Basidiomycetes have been identified at present, but only some of these are used in the human diet. Mushrooms are popular food products since ancient times not only because of the flavour, but also because of their high nutritional value. They are low in calories and fats and high in proteins, vitamins and minerals [1]. In addition, many beneficial properties of mushrooms have been described including antitumor, immunomodulation, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic efficacy [2]. Mushrooms are reported to have a preventing effect on diseases such as hypertension, hypercholesterolemia, atherosclerosis and cancer due to their specific chemical composition [3]. Important healing products can be isolated from both edible and non-edible mushrooms [1]. Today about 7000 species possess varying degrees of edibility; in addition, 2000 species have been suggested to possess medicinal properties. Such medicinal mushrooms produce substances that can improve biological functions and thus the health of the consumer [1].

Lipids are the least studied components in mushrooms since their overall content in the species known as yet is hardly higher than 6–8% of the dry weight. While the low lipid content is one of the advantages of mushrooms as nutrients, the knowledge of the composition of lipids as primary metabolites is of interest both because it adds to the general nutritional value and because it may characterize the special place of mushrooms in terrestrial life forms. Currently, studies of lipids are focused on the fatty acids (FA) as main structural lipid element (see [3–7] and the references cited therein). The knowledge of their identity and quantitative proportions provides important primary characteristics of the lipid components present. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) of fatty acid methyl esters (FAME) are the methods of choice so far [3,5–7]. The use of FAME, however, limits the direct localization of the double bonds position in the carbon chain [3,5–7] especially in complex FA samples. Even so, in some cases chemometric treatment of the mass spectral data has proved useful [8,9]. The fatty acid composition in some higher Basidiomycota species [4,10] (as picolinyl derivatives) and Lycoperdaceae species [11] (as 4,4-dimethyloxazoline derivatives – DMOX) has been reported and these are the most detailed

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Table 1
Fatty acids content of wild mushroom species of the Agaricales order determined by GC–MS of DMOX derivatives (rel.%).

Sample no.															
Fatty acid	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10:0	0.0[b]	0.0	0.0	0.0	0.1	0.0	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0
12:0	0.2	0.6	0.2	0.0	0.1	0.1	0.5	0.2	0.2	1.3	0.5	0.2	0.1	0.3	0.1
13:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14:0	0.8	0.7	0.4	0.3	0.3	0.3	2.1	0.5	0.4	1.1	0.1	0.5	0.5	0.6	0.3
15:0	1.0	0.5	0.9	0.5	0.5	0.4	0.6	0.3	0.7	0.4	0.7	0.7	0.1	1.4	0.3
16:0	15.8	16.7	16.5	14.6	12.5	13.8	23.1	22.0	18.9	18.0	16.3	16.1	15.9	16.3	17.9
6–16:1	0.0	0.1	0.5	0.7	0.7	0.2	0.4	1.3	0.2	0.3	0.2	0.8	0.1	2.1	0.2
9–16:1	2.6	0.5	0.6	0.8	0.5	0.8	1.1	1.4	1.1	0.4	0.7	2.0	0.4	3.7	0.3
11–16:1	0.1	0.4	0.2	0.2	0.3	0.6	0.0	0.1	0.2	0.4	0.3	0.5	0.4	1.4	0.2
17:0	0.1	0.4	0.6	0.5	0.5	0.3	0.4	0.3	0.1	0.2	0.2	0.2	0.1	0.9	0.1
7,10–16:2	0.1	0.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
9,12–16:2	0.0	0.0	0.3	0.6	0.5	0.1	0.0	0.1	0.2	0.4	0.0	0.4	0.0	0.6	0.0
18:0	4.9	5.4	5.6	4.6	4.9	1.7	20.3	7.5	3.2	5.3	8.1	6.6	0.2	5.3	1.8
11,12-M[c]-18:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.9
9–18:1	6.6	2.4	3.2	9.6	8.0	14.5	16.9	42.7	33.4	16.4	33.4	6.7	19.6	38.7	15.4
11–18:1	0.0	1.2	0.0	0.0	0.0	1.6	0.8	0.4	0.8	4.9	0.0	4.1	1.0	1.3	3.5
9,12–18:2	58.5	59.8	62.3	59.8	61.4	62.9	30.9	18.5	38.8	49.0	35.4	58.8	58.6	22.8	56.7
6,9,12–18:3	0.0	0.0	0.0	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:0	2.8	6.2	3.8	1.1	1.5	0.1	0.5	0.2	0.3	0.1	0.6	0.2	0.1	0.3	0.1
9,12,15–18:3	0.1	0.1	0.2	0.4	0.3	0.1	0.1	0.0	0.1	0.1	0.0	0.4	0.2	0.0	0.0
9–20:1	0.0	0.0	0.2	0.5	0.3	0.1	0.1	0.0	0.1	0.1	0.3	0.0	0.0	0.4	0.1
11–20:1	0.1	0.1	0.0	0.5	0.6	0.1	0.1	0.4	0.1	0.1	0.2	0.1	0.0	0.2	0.1
21:0	0.6	0.4	0.3	0.5	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
9,12–20:2	0.1	0.1	0.1	0.2	0.3	1.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	1.2
22:0	3.6	3.4	2.3	2.4	3.5	0.1	0.0	1.2	0.3	0.3	0.6	0.2	0.6	0.1	0.1
13–22:1	0.1	0.0	0.3	0.0	0.1	0.4	0.4	0.9	0.0	0.0	1.3	0.0	0.0	1.1	0.0
23:0	0.3	0.1	0.2	0.3	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.2	1.1	0.0
24:0	1.1	0.5	1.0	1.3	1.1	0.0	0.1	1.0	0.4	0.6	0.5	0.5	0.1	0.0	0.4
15–24:1	0.1	0.0	0.0	0.2	0.1	0.3	0.7	0.5	0.2	0.0	0.3	0.4	0.0	0.2	0.1
25:0	0.1	0.0	0.0	0.0	0.3	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.7	0.0
26:0	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
ΣU[d]	68.4	65.0	68.1	73.9	73.5	82.7	51.7	66.5	75.3	72.4	72.3	74.5	81.9	72.6	78.8
U/S[e]	2.2	1.9	2.1	2.8	2.8	4.8	1.1	2.0	3.0	2.6	2.6	2.9	4.5	2.7	3.7
L/O[e]	8.9	25.3	19.3	6.2	7.6	4.3	1.8	0.4	1.2	3.0	1.1	8.7	3.0	0.6	3.7

[a] Samples numbers as in Table 2.
[b] Each value is mean of three measurements with RSD not exceeding 7.0%.
[c] Methyl branched group.
[d] Sum of the unsaturated fatty acids.
[e] U: unsaturated, S: saturated, L: linoleic, O: oleic fatty acids.

data on the fatty acid composition of mushrooms published so far. The chemical composition and nutritional value of wild species of mushrooms growing in Europe have been recently thoroughly reviewed [12].

The classification of fungi is usually performed by morphological criteria. Recently, fatty acid composition showed increased potential for characterization, differentiation and sorting of fungal samples. Using the composition of basic chemical compounds in addition to the conventional morphological criteria was found to be very helpful in the characterization and classification of unknown species and in cases of doubtful identification. Fatty acids composition, combined with methods of multivariate statistical analysis, has been successfully used for some fungi classification on limited number of species [13–16].

Using GC–MS of DMOX derivatives we determined the fatty acid composition of 15 mushroom species of the order Agaricales which is the most diverse order in the phylum Basidiomycota, with 33 families, 413 genera and over 13,000 described species [17]. The aim of this work was to investigate whether fatty acid composition reflects the classification of the chosen mushroom species in a reliable way. The samples were firstly identified by the usual morphological approach and then chemometrics was applied to the fatty acid composition to expose these differences that are in agreement with the classification.

The result of this work is presented here.

2. Materials and methods

2.1. Reagents and samples

All reagents and solvents were of analytical grade. Hexane was left for 24 h over potassium hydroxide and then distilled; diethyl ether was peroxide-free.

The mushrooms were collected during the summer and autumn of 2006 and 2007 at the foot of the Rila Mountain in the Southwest of Bulgaria. All species belong to the order Agaricales and are specified in Table 1. Our preliminary experiments with two different collections of *Agaricus campestris* and *Marasmius oreades* had shown that specimens of the same species, but of different growing stage, differed significantly in fatty acid composition. On the other hand, another set of four collections of specimens of the same species as above showed that the differences in fatty acid composition of mushrooms collected in two consecutive years were insignificant. Thus, each sample in this work represented 6–8 specimens of each species, of the same growing stage and of the same habitat (the one typical for the respective species). Non edible mushrooms were also included in our investigation to find out whether their fatty acid composition was significantly different from composition of the edible species. Immediately after gathering (gloves handling) the fruit body of each specimen was cleaned carefully from grass, soil and other material with a dry soft bristle brush and was then

Table 2
Wild mushroom species of the Agaricales order and their lipid content.

Sample no.	Species	Family	Lipid content (% of dry weight)
1	*Agaricus augustus*	*Agaricaceae*	4.0 ± 0.1[a]
2	*Agaricus arvensis*	*Agaricaceae*	4.0 ± 0.2
3	*Agaricus silvaticus*	*Agaricaceae*	6.1 ± 0.2
4	*Agaricus campestris*	*Agaricaceae*	3.0 ± 0.1
5	*Agaricus xanthoderma v. meleagris*[*]	*Agaricaceae*	4.0 ± 0.2
6	*Endoptychum agaricoides*	*Agaricaceae*	3.5 ± 0.1
7	*Marasmius oreades*	*Marasmiaceae*	5.1 ± 0.2
8	*Hygrocybe nigrescens*[*]	*Tricholomataceae*	6.0 ± 0.3
9	*Clitocybe clavipes*[*]	*Tricholomataceae*	4.0 ± 0.2
10	*Lyophyllum connatum*	*Tricholomataceae*	4.0 ± 0.1
11	*Lyophyllum decastes*	*Tricholomataceae*	4.1 ± 0.2
12	*Lepista gilva*	*Tricholomataceae*	5.1 ± 0.2
13	*Lepista inversa*	*Tricholomataceae*	6.7 ± 0.2
14	*Pluteus atricapillus*[*]	*Plutaceae*	9.0 ± 0.3
15	*Pleurotus dryinus*	*Pleurotaceae*	6.7 ± 0.2

[a] Mean ± SD of two measurements.
[*] The asterisk indicates the species that are either toxic (*Agaricus xanthoderma*) or not commonly used because of the specific taste or odour.

placed in a glass container filled with hexane in order to prevent enzymatic degradation and autoxidation of lipids [18]. The containers were transported to the laboratory the same day and immediate isolation of lipids was carried out.

2.2. Determination of the lipid content

When delivered to the lab, the hexane layer was decanted since (i) according to our preliminary tests by thin layer chromatography (5 cm × 20 cm silica gel G plates; mobile phase hexane–acetone 100:8 (v/v), reference mixture containing phospholipids, free fatty acids, monoacylglycerols, diacylglycerols, sterols, triacylglycerols, fatty acid methyl esters and sterol esters) it did not contain even traces of any lipid component and (ii) in presence of hexane it was very difficult to destroy the emulsions formed (usually) at the subsequent extraction steps.

The procedure described by Christie [18] was applied. The fruit body was cut into small pieces, briefly blended with methanol (10 mL/g tissue) and stirred for 20 min. Then, the sample volume was doubled by adding chloroform and the mixture was stirred for additional 4 h. The combined organic layers were evaporated, and the crude extract was taken up in about 24 mL of fresh mixture of chloroform-methanol, 2:1 (by volume) and subjected to a "Folch" wash (with ¼ of the total volume, e.g. 6 mL of 0.88% potassium chloride in water) in order to eliminate non-lipid contaminants. The aqueous (upper) layer, containing non-lipid contaminants, was removed. The organic layer, which contained the purified lipids, was washed with the same volume (as the upper layer) of methanol–saline solution, 1:1 (by volume). The bottom layer was transferred to a round bottom flask and the major part of the solvent was evaporated on a rotary evaporator. The rest was quantitatively transferred to a pre-weighed glass vial using chloroform–methanol, 2:1 (by volume). The solvent was evaporated under gentle stream of nitrogen to a constant weight, which was related to the weight of the thoroughly vacuum-dried at 60 °C mushroom residue in order to determine the percentage of total lipid content in dry material. Lipids were dissolved in hexane to give a 5% stock solution and kept at −20 °C until analyzed.

2.3. Preparation of DMOX derivatives

The procedure described by Christie [18] was followed with small modifications. Initially, the lipid sample (up to 10 mg) was hydrolyzed to free fatty acids by heating for 3 h at 50 °C with freshly prepared 0.1 M potassium hydroxide in 90% aqueous ethanol (0.25 mL per mg sample). Then, 2 M hydrochloric acid (0.05 mL per mg sample), 3 mL hexane, 3 mL diethyl ether and 2 mL water were added and the mixture was vigorously shaken. The upper organic layer was taken with a Pasteur pipette and was passed through a short column with anhydrous sodium sulfate. The solvents were evaporated under a gentle stream of nitrogen, the free fatty acids (up to 2 mg) were immediately subjected to reaction with 2-amino-2-methyl-1-propanol (0.25 g) at 190 °C for 4 h in a closed sample vial, preliminary flushed with nitrogen to eliminate moisture and minimize autoxidation. After cooling, 5 mL *n*-hexane–diethyl ether, 1:1 (by volume) were added followed by 5 mL of water. The organic layer was transferred to a test tube and was dried over anhydrous sodium sulfate for 1 h. The solution was then taken with a Pasteur pipette and passed through a small column of anhydrous sodium sulfate into pre-weighed vial. The solvent was evaporated under stream of nitrogen and the residue was dissolved in *n*-hexane to give a 2% solution of DMOX derivatives. Thorough drying of the sample gives a more stable product. The completeness of the reaction was checked by silica gel TLC with a mobile phase of 3 mL chloroform–methanol, 100:3 (by volume) to a front of 10 cm. DMOX derivatives (R_f = 0.4) were detected by treating the plate with 5% ethanolic sulphuric acid and heating at 180 °C on a temperature controlled hot plate.

2.4. Analysis of DMOX derivatives by GC–MS

An Agilent 6890 Plus System (Agilent Technologies, Santa Clara CA, USA) equipped with a 5793 mass selective detector (Agilent Technologies, Santa Clara CA, USA) and a 30 m × 0.25 mm × 0.25 μm SP 2380 capillary column (Supelco, Bellefonte PA, USA) was used to examine the fatty acids DMOX derivatives. The temperature gradient started from 150 °C with 3 °C/min to 230 °C and held at this temperature for 15 min; solvent delay was 2.2 min, T_{inj} was 260 °C and T_{aux} – 280 °C. Helium was the carrier gas at 0.8 mL/min. The mass detector operated at T_{quad} 150 °C and T_{source} at 230 °C. Injection volume was 1.5 μL; split 20:1. Limit of quantification was 0.03 rel.%.

2.5. Multivariate analysis

The matrix of individual fatty acid contents for each mushroom (Table 1) consists of 15 objects (mushrooms) and 30 variables (fatty acids). In order to illustrate the correlation patterns among the mushrooms and the fatty acids, Principal Component Analysis (PCA) [19] was performed on this data matrix. This was done on autoscaled data due to the varying range of the fatty acids. PCA is a common tool used to extract information from a data matrix, and

the resulting score and loading plots are presented and interpreted in Section 3.

All multivariate data analysis was done using Sirius version 8.5, available from Pattern Recognition Systems AS (www.prs.no), Bergen, Norway.

3. Results and discussion

3.1. Fat content

The fat content of different species of mushrooms is reported to range from 1.1 to 8.3% on a dry weight basis, with an average content of 4.0% [1]. The fat content of Agaricales mushrooms studied in this work is shown in Table 2 and is in general agreement with the values reported for other Basidiomycetes [4,5]. Fat content of 6.0–6.7% was determined in *Agaricus silvaticus*, *Hygrocybe nigrescens*, *Lepista inversa* and *Pleurotus dryinus* and unusually high content of 9% – in *Pluteus atricapillus*. The fat content did not exceed 4% in samples *Agaricus augustus*, *Agaricus arvensis*, *Agaricus xanthoderma v. meleagris*, *Endoptychum agaricoides*, *Clitocybe clavipes* and *Lyophyllum connatum*.

3.2. Fatty acid composition

GC is the method of choice when determining the fatty acid composition of a lipid sample and it works perfectly well with simple mixtures. Even though mushroom samples are considered low in fats, the constituent fatty acids, besides the few major ones, include series of minor components, suspected to be unsaturated isomers, odd-chain and long-chain saturated and monoenoic fatty acids. Described first in 1988 [20], DMOX derivatives were chosen in the present work because they have proved to (i) be easy to prepare; (ii) give unique and readily interpretable mass spectra, and (iii) have the same elution order as FAMEs and only about 10 °C higher elution temperature than the corresponding FAME [21]. The mass spectra of DMOX fatty acid derivatives show intensive fragments at *m*/*z* 113 and 126. For saturated fatty acids the *m*/*z* 126 is followed by a homologous series of 14 atomic mass units (amu). The double bond positions can be easily deduced by characteristic 12 amu breaks at the olefinic bond in the homologous 14 amu sequence of the saturated hydrocarbon chain. Two more intense peaks are explained by allylic cleavage on both sides on the double bond [22–24].

Thirty one fatty acids were identified in total, not all of which were present in all species (Table 1). They were identified by interpreting their mass spectra as DMOX derivatives according to published data [21–24] and comparison with reference spectra [25].

As has been found for other Basidiomycetes [3,4,7–11], linoleic, oleic and palmitic acids are the main fatty acids. The mean summary value of the three acids was found to be 83.7%, ranging between 70.9% for sample 7 (*Marasmius oreades*) to 91.2% for sample 6 (*Endoptychum agaricoides*).

The sum of unsaturated fatty acids in the studied samples accounted for an average of 71.8% of total FA, being at least 51.7% of total (sample 7), lower in samples 1, 2, 3, 7 and 8 and higher in samples 9, 12, 13 and 15 reaching highest value (82.7%) in sample 6. This resulted in mean value of 2.8 for the U/S ratio ranging from 1.1 (sample 7) to 4.8 (sample 6). The high content of unsaturated fatty acids is mainly due to linoleic acid (mean content of all samples 48.9%), which, accounts for 62.9% in sample 6, 62.3% in 3, 61.4% in 5, down to 35.4% for 11, 30.9% for 7, and just 22.8% for 14 and 18.5% for sample 8. Oleic acid (9–18:1) has low content (under 10%) in samples 1 to 5 and 12, a mean content of 17.8% (samples 7, 10, 13, 15) and high content (over 30%) in samples 8, 9, 11, 14.

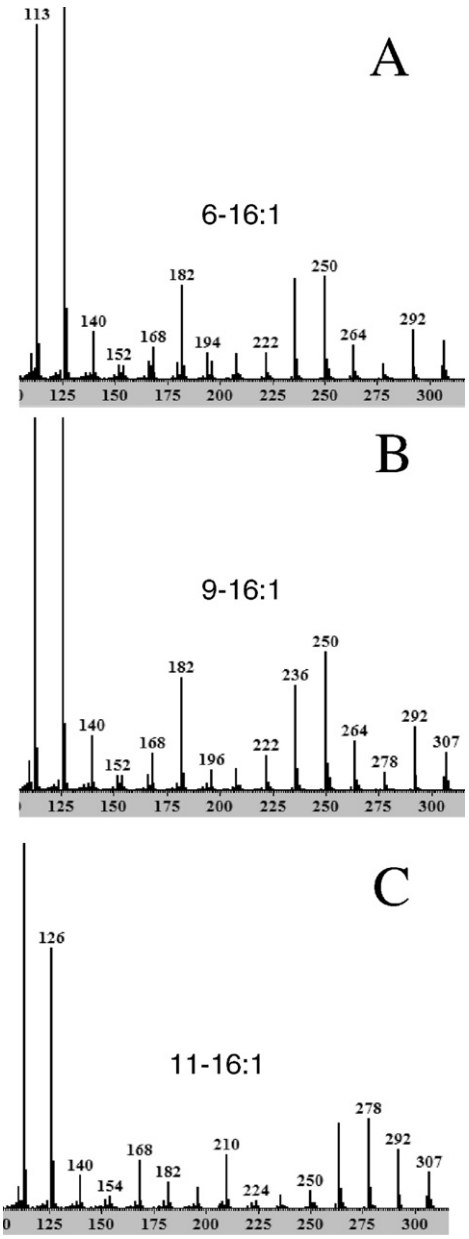

Fig. 1. Mass spectra of the three isomeric 16:1 acids in *Pluteus atricapillus* (sample 14 as shown in Table 2). A, 6–16:1; B, 9–16:1; C, 11–16:1.

Consequently, the L/O ratio is higher (about 20 and over) in samples 2 and 3, gradually decreasing (between 9 and 3) in samples 1, 12, 4, 5, 6, 10 and 13, respectively, roughly equals 1 (samples 7, 9, 11) and reaches 0.5 (samples 8 and 14). The polyenoic/monoenoic fatty acids ratio follows the same tendency. Nutritionally important linolenic acid isomers were found in minor amounts (up to 0.4%) – all *cis* 6,9,12-18:3 (γ-linolenic acid or ω-6 isomer) was found only in samples 4 and 5 (Agaricus) while the α-isomer (all *cis* 9,12,15-18:3 or ω-3 isomer) was found in eleven of the samples.

Since unsaturated fatty acids are essential in the human diet [26] the finding of a high proportion of unsaturated fatty acids and, especially, the high percentage of linoleic acid in these mushrooms is a significant factor in regarding mushrooms as a health food.

In confirmation with a previous work on fatty acid composition of other Agaricales mushrooms [11], three isomeric 16:1 fatty acids (with double bond in positions 6-, 9-, and 11-) were fully resolved, identified and quantified, thus allowing to assume that these minor components are typical for the order. Their sum varied between

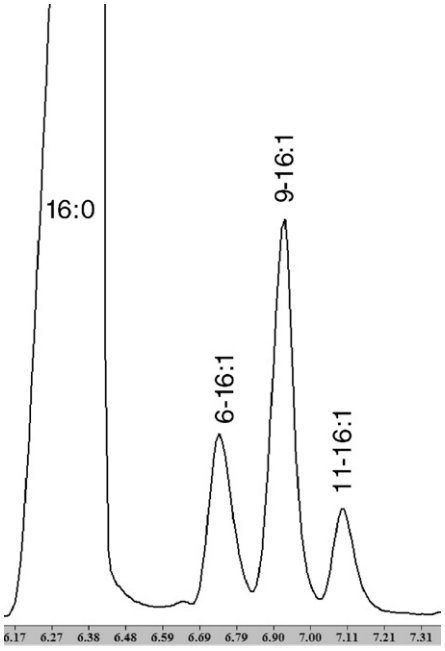

Fig. 2. Total ion chromatogram (TIC) of 16:0 + the three 16:1 isomers, separated as DMOX derivatives, in *Pluteus atricapillus* (sample 14 as shown in Table 2).

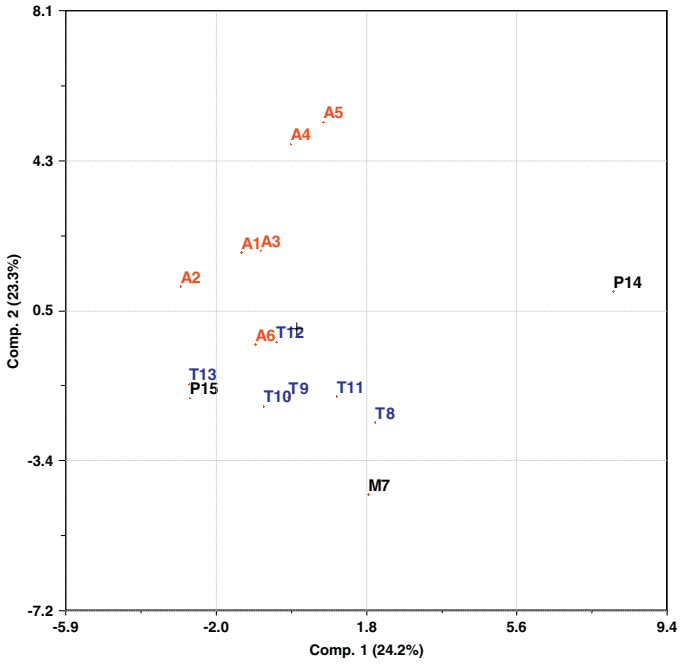

Fig. 3. Score plot of the first two score vectors of the autoscaled fatty acid data from Table 1. Each sample is represented by a letter and a number. Letters correspond to the families: A – *Agaricaceae*, T – *Tricholomataceae*, M – *Marasmiaceae*, P – *Plutaceae* and *Pleurotaceae* mushrooms. The number used refers to the numbering in Table 2.

0.7% (sample 15) and 7.2% (sample 14). Fig. 1 shows the mass spectra of the three isomeric 16:1 acids. The base peak in the spectrum of the DMOX derivative of the 6-isomer is that at $m/z = 126$, the position 6 of the double bond was evident by the relatively abundant ions at m/z 167 and 194 (Fig. 1A). The locations of the double bonds in positions 9- and 11- were revealed by the 12 amu gaps at m/z 196–208 and m/z 224–236, respectively, as shown in Fig. 1B and C. Separation of the three 16:1 isomers could be seen in Fig. 2.

3.3. Chemometrics

The first two principal components (PCs) explained 48% of the variance of the data, and the resulting score plot is shown in Fig. 3. Each sample is represented by a letter and a number. The letter 'A' is used for the *Agaricaceae* mushrooms. The letter 'T' is used for the *Tricholomataceae* family of mushrooms. The letter 'M' is used for the *Marasmiaceae* mushroom. The letter 'P' is used for the *Plutaceae* and the *Pleurotaceae* mushrooms. The number used refers to the numbering in Table 2. The most striking feature of Fig. 3 is how different *Pluteus atricapillus* (sample 14, one of the inedible ones) is from the others. We also observe that the *Agaricaceae* mushrooms (samples 1–6) cluster, as do the *Tricholomataceae* mushrooms (samples 8–13). The only significant deviation from this is the mushroom species *Endoptychum agaricoides* (genus *Endoptychum*, sample 6) clearly pointing that it belongs to another genus and whose position in the plot close to the *Tricholomataceae* mushrooms indicates a resemblance to these with regards to the fatty acid profile.

The loading plot in Fig. 4 explains the nature of these similarities and differences. Thus, the outlying sample 14 is low in polyunsaturated FA, and it has an uncharacteristically high content of the fatty acids to the right in the figure, such as monounsaturated 6–16:1, 9–16:1, 11–16:1, 13–22:1 and 9–18:1, and the saturated 25:0, 15:0, 17:0 and 23:0.

The presence of the outlying *Plutaceae* mushroom masks the similarities and differences among the other mushrooms, and the analysis was therefore redone without said mushroom (sample 14). The resulting score and loading plots are shown in Figs. 5 and 6, respectively. The letters and numbers used in the score plot (Fig. 5) are the same as in Fig. 3. The tendency of mushrooms of the same family to group together is still evident, indicating that the fatty acid composition is different for different mushroom families. From the positioning of the objects in the score plot and the variables in the loading plot the most striking difference between the *Agaricaceae* (samples 1–6) and the *Tricholomataceae* (samples 8–13) mushrooms is that the former have a higher content of the polyunsaturated and long chained saturated fatty acids. The *Tricholomataceae* mushrooms are to a larger extent characterized by a higher content of short chained saturated fatty acids. The score plot

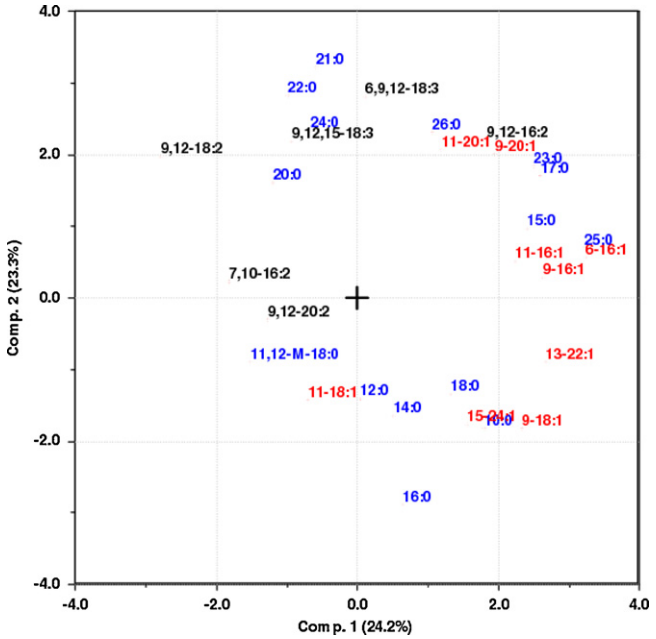

Fig. 4. Loading plot of the first two loading vectors of the autoscaled fatty acid data from Table 1.

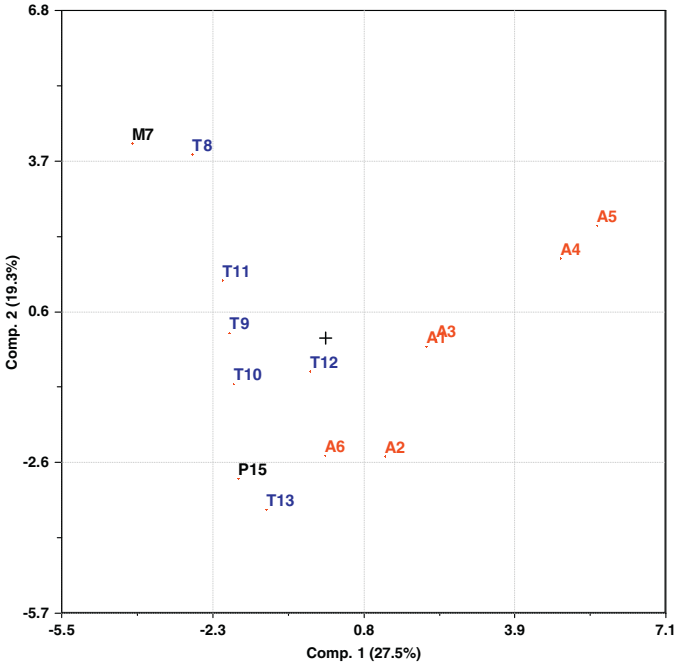

Fig. 5. Score plot of the first two score vectors of the autoscaled fatty acid data from Table 1. Sample 14 excluded from the analysis. The same coding is used as in Fig. 3.

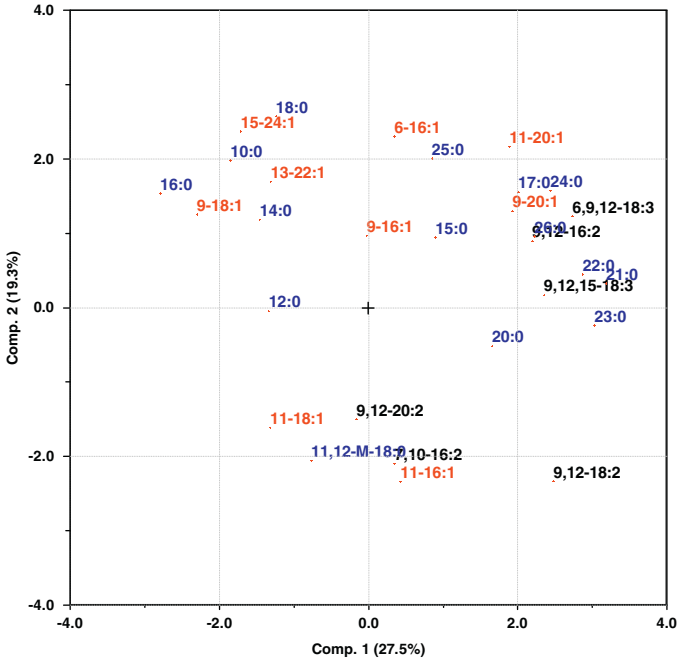

Fig. 6. Loading plot of the first two loading vectors of the autoscaled fatty acid data from Table 1. Sample 14 excluded from the analysis.

(Fig. 5) shows that the *Agaricaceae* mushrooms (objects 1–6) have a positive score for the first component, whereas the *Tricholomataceae* mushrooms (objects 8–13) have a negative score. The first principal component is able to discriminate between the two types of mushrooms based on the fatty acid composition. A bar plot of the loadings (Fig. 7) elucidates the reasons for this discrimination. The polyunsaturated fatty acids 7,10–16:2, 9,12–16:2, 9,12–18:2, 6,9,12–18:3 and 9,12,15–18:3 have a positive loading. The same applies for the longer chained saturated fatty acids 20:0, 21:0, 22:0, 23:0, 24:0, 25:0 and 26:0, which are towards the right of the plot. These fatty acids are characteristic for the *Agaricaceae* mushrooms, which had a positive score for the component. The short chained saturated fatty acids 10:0, 12:0, 14:0, 16:0 and 18:0 are in the left end of the plot. Their loadings are negative, corresponding to the situation for the *Tricholomataceae* mushrooms.

It is of course tempting to try to use more advanced chemometrics techniques for classification and discrimination, such as SIMCA [27] and PLS discriminant analysis [28]. Such models are not presented in this paper, as the number of samples in the dataset is too small to warrant the use of these more advanced techniques.

4. Conclusion

The data obtained in this work adds to the scarce information on the very complex and unique fatty acid composition of mushrooms, including new species to these examined before.

The fatty acid composition of new Agaricales families and genera confirms the fact that the fatty acid composition of mushrooms is rather complex. Besides components, which are most abundant for terrestrial organisms – palmitic, oleic and linoleic acids, longer chain saturated and unsaturated fatty acids, including positional isomers, were identified. Evidently, in general, the fatty acid composition is not affected strongly either by the location or by the year of growing. While some information may be extracted from the data material by simply looking at it, chemometrics provides approach elucidating more clearly the major correlation patterns

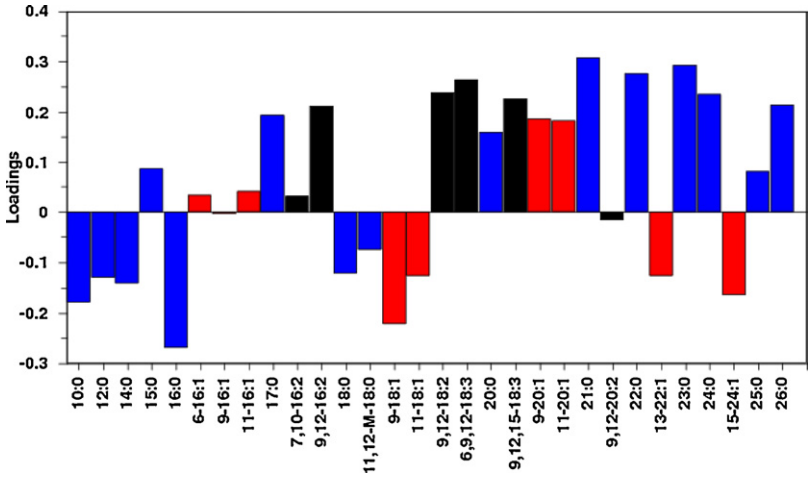

Fig. 7. The first loading vector against the variables after outlier removal.

with regards to the fatty acid distributions among the mushrooms species, genera and families. The groupings in the score plot indicate that the fatty acid composition reflects the biological classification. Thus, it is possible to distinguish between different families of mushrooms. A more thorough study of the score and loading vectors showed that the *Agaricaceae* family contains more of the longer straight-chain saturated fatty acids than the *Tricholomataceae* family. Examination of more *Plutaceae* and *Pleurotaceae* species will help to see more clearly the similarity and differences in the Agaricales families in regards to the fatty acid composition.

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References

[1] S.T. Chang, P.G. Miles, in: S.T. Chang, P.G. Miles (Eds.), Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, 2nd ed., CRC Press, Boca Raton, FL, USA, 2004, p. 27.
[2] S.P. Wasser, A.L. Weis, Int. J. Med. Mushrooms 1 (1999) 31.
[3] N. Yilmaz, M. Solmaz, I. Turkekul, M. Elmastas, Food Chem. 99 (2006) 168.
[4] K. Pedneault, P. Angers, A. Gosselin, R.J. Tweddell, Mycol. Res. 110 (2006) 1179.
[5] V.A. Diez, A. Alvarez, Food Chem. 75 (2001) 417.
[6] I. Brondz, K. Hoiland, D. Ekeberg, J. Chromatogr. B 800 (2004) 303.
[7] T. Rezanka, P. Mares, J. Chromatogr. 409 (1987) 390.
[8] F. Brakstad, Chemom. Intell. Lab. Syst. 19 (1993) 87.
[9] S.A. Mjos, Eur. J. Lipid Sci. Technol. 106 (2004) 550.
[10] K. Pedneault, P. Angers, A. Gosselin, R.J. Tweddell, Mycol. Res. 112 (2008) 1428.
[11] D. Nedelcheva, D. Antonova, S. Tsvetkova, I. Marekov, Sv. Momchilova, B. Nikolova-Damyanova, M. Gyosheva, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 2717.
[12] P. Kalac, Food Chem. 113 (2009) 9.
[13] G. Blomquist, B. Andersson, K. Andersson, I. Brondz, J. Microbiol. Methods 16 (1992) 59.
[14] P.D. Stahl, M.J. Klug, Appl. Environ. Microbiol. 62 (1996) 4136.
[15] S.V. Diehl, M. Lynn Prewitt, F.M. Shmulsky, ACS Symp. Ser. 845 (2003) 313.
[16] M.E. Fraga, D.M.N. Santana, M.J. Gatti, G.M. Direito, L.R. Cavaglieri, C.A.R. Rosa, Mem. Inst. Oswaldo Cruz 103 (2008) 540.
[17] P.M. Kirk, P.F. Cannon, D.W. Minter, J.A. Stalpers, Dictionary of the Fungi, 10th ed/, CAB International, Wallingford, England, 2008, p. 12.
[18] W.W. Christie, Lipid Analysis, 3rd ed., The Oily Press, Bridgwater, England, 2003.
[19] I.T. Joliffe, Principal Component Analysis, 2nd ed., Springer, NY, 2002.
[20] J.Y. Zhang, Q.T. Yu, B.N. Liu, Z.H. Huang, Biomed. Environ. Mass Spectrom. 15 (1988) 33.
[21] G. Dobson, W.W. Christie, Eur. J. Lipid Sci. Technol. 104 (2002) 36.
[22] L. Fay, U. Richli, J. Chromatogr. 541 (1991) 89.
[23] V. Spitzer, Prog. Lipid Res. 35 (1997) 387.
[24] W.W. Christie, G.W. Robertson, W.C. McRoberts, J.T.G. Hamilton, Eur. J. Lipid Sci. Technol. 102 (2000) 23.
[25] W.W. Christie, 4,4-Dimethyloxazoline (DMOX) Derivatives of Fatty Acids – Archive of Mass Spectra, at <http://lipidlibrary.aocs.org/ms/arch_dm/index.htm> (accessed December 2011).
[26] G.D. Lawrence, The Fats of Life: Essential Fatty Acids in Health and Disease, Rutgers University Press, Piscataway, NJ, 2010.
[27] S. Wold, Pattern Recogn. 8 (1976) 127.
[28] M. Barker, W. Rayens, J. Chemom. 17 (2003) 166.